Increases in lung expansion alter pulmonary hemodynamics in fetal sheep

Graeme R. Polglase, Megan J. Wallace, David L. Morgan, and Stuart B. Hooper. Increases in lung expansion alter pulmonary hemodynamics in fetal sheep. J Appl Physiol 101: 273–282, 2006. First published March 30, 2006; doi:10.1152/japplphysiol.01544.2005.—Pro-longed increases in fetal lung expansion stimulate fetal lung growth and development, but the effects on pulmonary hemodynamics are unknown. Our aim was to determine the effect of increased fetal lung expansion, induced by tracheal obstruction (TO), on pulmonary blood flow (PBF) and vascular resistance (PVR). Chronically catheterized fetal sheep (n = 6) underwent TO from 120 to 127 days of gestation age (term ~147 days); tracheas were not obstructed in control fetuses (n = 6). PBF, PVR, and changes to the PBF waveform were determined. TO significantly increased lung wet weight compared with control (166.3 ± 20.2 vs. 102.0 ± 18.8 g; P < 0.05). Despite the increase in intraluminal pressure caused by TO (5.0 ± 0.9 vs. 2.4 ± 1.0 mmHg; P < 0.001), PBF and PVR were similar between groups after 7 days (TO 28.1 ± 3.2 vs. control 34.1 ± 10.0 ml·min⁻¹·100 g lung wt⁻¹). However, TO markedly altered pulmonary hemodynamics associated with accentuated fetal breathing movements, causing a reduction rather than an increase in PBF at 7 days of TO. To account for the increase in intraluminal pressure, the pressure was equalized by draining the lungs of liquid on day 7 of TO. Pressure equalization increased PBF from 36.8 ± 5.2 to 112.4 ± 22.8 ml/min (P = 0.01) and markedly altered the PBF waveform. These studies provide further evidence to indicate that intraluminal pressure is an important determinant of PBF and PVR in the fetus. We suggest that the increase in PBF associated with pressure equalization following TO reflects an increase in growth of the pulmonary vascular bed, leading to an increase in its cross-sectional area.

fetal breathing movements; Valsalva maneuvers; pulmonary blood flow

FETAL LUNG GROWTH AND DEVELOPMENT is largely determined by the degree to which the fetal lungs are expanded by liquid. As this liquid is secreted by the lungs and exits via the trachea (5, 12, 27, 35, 36), obstruction or ligation of the fetal trachea prevents liquid efflux, causing it to accumulate within the future airways. The resulting increase in lung expansion markedly accelerates lung cell proliferation, stimulates alveolar formation, enhances thinning of the future alveolar walls, and promotes type II to type I alveolar epithelial cell (AEC) transdifferentiation. On the other hand, fetal lung deflation causes lung development to cease, resulting in lungs that are hypoplastic, deficient in alveoli, but with increased proportions of type II AECs (5, 12, 35). However, little is known of the effects of fetal tracheal obstruction (TO) on the pulmonary vasculature and hemodynamics, although it is known that TO stimulates endothelial cell proliferation in the fetal lung (37).

During fetal development, pulmonary vascular resistance (PVR) is high (23, 25), and only ~12% of right ventricular output passes through the lungs. Most bypasses the fetal lungs and enters the systemic circulation via the ductus arteriosus (DA) (13, 24, 44, 46, 49). In the fetus, a high PVR and the presence of a patent DA confer unique characteristics to the contour of the PBF waveform, which rapidly disappears at birth (46). This waveform is characterized by a large negative component (retrograde flow) during diastole when blood flows away from the lungs and exits the pulmonary circulation via the DA (46). The high PVR in the fetus is believed to be due to the low Po₂ of arterial blood (30, 49), the balance between vasoconstrictor and vasodilator activity (7, 24, 42, 54), as well as a high level of resting lung expansion and intraluminal pressure (25, 53). Intraluminal pressures, caused by changes in lung expansion, have a major impact on fetal pulmonary blood flow (PBF), as they do in the newborn (14, 15, 40) and adult (22, 39, 51). Increases in intraluminal pressure reduce PBF, whereas reductions in intraluminal pressure increase PBF and reduce PVR (25, 40, 53). Similarly, the reductions in intratra-checal pressure associated with accentuated fetal breathing movements (FBM), as well as deep inspiratory efforts, cause large, transient increases in PBF (41).

Although TO is a potent stimulus for fetal lung growth, the associated increase in intraluminal pressure [to 5–6 mmHg (36)] would be expected to further increase PVR and reduce PBF. However, a reduction in PBF is not consistent with the large increase in lung growth induced by TO (27, 36), although the additional metabolic requirements of this growth response are unknown. In fetuses with pulmonary hypoplasia, prolonged TO reduces the pulsatility index (PI) of flow within the pulmonary artery, suggesting that increased lung expansion can reduce the resistance to pulsatile flow in small pulmonary vessels, perhaps by increasing pulmonary vascular growth (47). This is supported by the finding that TO stimulates endothelial cell proliferation within the perialveolar region of the lung (37). Our aim was to determine the longitudinal changes in PBF during 7 days of TO and to determine whether PBF increases in proportion with the increase in lung growth. We hypothesized that PBF would remain unaltered during TO, despite the increase in intraluminal pressure. Furthermore, we hypothesized that the relationship between PBF and accentuated FBMs would reverse following TO, when the intratracheal pressure changes associated with FBM reverse. Our laboratory has previously shown that, after ~4 days of TO, the fetal diaphragm everts (due to the expanding lung), and, therefore, contraction of the fetal diaphragm causes compression and not expansion of the fetal lung (27). As a result, the intratracheal

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pressure fluctuations associated with FBM reverse and become positive.

Characteristics of the PBF waveform have been shown to be sensitive indicators of downstream resistance in the pulmonary vascular bed of ventilated preterm lambs (20, 40). Thus another aim was to determine the effect of changes to intraluminal pressure during TO on the PBF waveform contour and to identify the key components most sensitive to intraluminal pressure changes in the fetus.

METHODS

Aseptic surgery was conducted on 12 pregnant ewes (Border-Leicester × Merino) at 115–118 days of gestation (term is ~147 days), as described previously (41). Briefly, anesthesia was induced using thiopentone sodium (1 g iv) and was maintained, after tracheal intubation, with 1.5% halothane in O2; antibiotics (1 g iv ampicillin) were administered to the ewe on induction of anesthesia. Polyvinyl catheters were implanted into the fetal carotid artery, jugular vein, amniotic sac, and pulmonary artery, and a 4-mm ultrasonic flow probe (Transonic Systems, Ithaca, NY) was placed around the left main pulmonary artery. Two large-bore silicone rubber cannulas (Dow Corning) were implanted 2–4 cm into the fetal trachea: one directed toward the lungs, and the other directed toward, but not entering, the larynx (26). These catheters were joined externally to form a tracheal loop that allowed the continuous flow of lung liquid. All catheters and the ultrasonic flow transducer cable were externalized through the right flank of the ewe. Ewes and fetuses were allowed at least 3 days to recover from surgery, during which time fetuses were treated daily with thiopentone sodium (1 g iv) and was maintained, after tracheal intubation, with 1.5% halothane in O2; antibiotics (1 g iv ampicillin). Fetal well-being was monitored regularly by measuring fetal arterial blood PO2 (PaO2), arterial PCO2, pH, and percentage of O2 saturation of hemoglobin (ABL30, Radiometer).

Experimental Protocol

Fetuses were divided into two groups, and control recordings were made from all fetuses before commencing the experiment (for 6 h at 120 days gestational age). At 121 ± 1 days of gestation, fetuses in one group (n = 6) underwent TO for 7 days, whereas the trachea of control fetuses (n = 6) was not obstructed. Recordings were made for 6 h each day for 7 days between 9 AM and 5 PM, commencing ~1 h after the ewe had been fed to reduce the variability associated with circadian rhythms and the effect of maternal feeding on FBM (6). Fetel systemic and pulmonary blood pressures, tracheal pressure (Pt) (all pressures were normalized by subtraction of amniotic fluid pressure), blood flow through the left pulmonary artery, and heart rate (HR) were measured continuously and recorded electronically throughout the experiment (Powerlab/SP, ADInstruments, Castle Hill, Australia). On day 7 of TO, intraluminal pressure in TO fetuses was restored to values observed in control fetuses at the corresponding gestational age (~1–2 mmHg) by draining fetal lung liquid until the desired Pt was obtained. This level of Pt, was maintained for 2 h before the lung liquid was restored; the estimated volume of liquid remaining within the lungs of TO animals after intraluminal pressure restoration was ~45 ml/kg (36). Before autopsy, all fetuses were passively drained of lung liquid before the ewe and fetus were killed by an overdose of sodium pentobarbitone administered to the ewe (130 mg/kg iv). The fetus was weighed before the lungs were removed and weighed. Estimations of fetal body weight throughout the experiment were calculated as previously described (34). Total DNA content and concentration of the fetal lungs were determined using a bis benzamide fluorometric DNA assay (Hoechst 33258; Calbiochem). All experimental procedures on animals were approved by the Monash University Committee for Ethics in Animal Experimentation.

Analytical Methods

Each 6-h recording block was broken into episodes of FBM and apneaa. Episodes of FBMs were identified and categorized into accentuated and nonaccentuated FBMs, as previously described (41). All measurements made within a FBM episode were compared with the period of apnea immediately preceding the FBM episode to account for gestational age-related increases in PBF and other non-FBM-specific factors that may affect PBF. Valsalva-like maneuvers were identified as large increases in Pt (>2 mmHg change), which lasted for longer than 5 s in duration periods of apnea. All measurements made during Valsalva-like maneuvers were compared with the period of apnea immediately preceding the Valsalva-like maneuver.

Measurements of mean PBF and pulse-by-pulse minimum and maximum values of PBF were electronically computed from the PBF waveform and were rapidly calculated at ~2–3 Hz, as were pressures in the trachea (Pt) and pulmonary artery (Ppa). PVR was calculated using the formula PVR = (Ppa − Pla)/PBF, where Pla is left atrial pressure and is assumed to be 3 mmHg. In preliminary studies, Pla was found not to change significantly, despite the increase in intraluminal pressure, up to 5 days following the initiation of TO. All transducers and the blood flowmeter were balanced and/or zeroed every day before the recordings began.

Waveform Analysis

The changes in the contour of the PBF waveform were measured by selecting representative waveforms throughout 10 continuous cardiac cycles from each fetus during the control recordings, just before lung liquid drainage (7 days TO) and at 5-min intervals during and following Pt equalization. The waveform parameters examined include pulse amplitude, rate of pulse increase, rate of pulse decrease, pulse maximum, pulse minimum, postpulse minimum, end-diastolic flow, as well as the maximum, minimum, and average flow during the interpulse period, as previously described (40).

PI was calculated based on the method of Gosling and King (19) using the equation:

\[
PI = \frac{(Q_{max} - Q_{min})}{Q_{max}}
\]

where \(Q_{max}\) is peak systolic flow, \(Q_{max}\) is minimum diastolic flow, and \(Q_{max}\) is mean maximum flow averaged over several cycles.

Statistical Methods

A minimum of 160 measurements were obtained during FBM from each fetus (~20 per day) during the 8-day recording period, with similar numbers of measurements obtained during accentuated and nonaccentuated FBMs. To determine the effect of accentuated FBMs, measurements of PBF and PVR were made during accentuated FBMs and expressed as a percentage of the preceding period of apnea to account for gestational age-related and nonspecific changes in PBF and PVR, as previously described (41). To determine the temporal changes in PBF and PVR, for each fetus, the mean PBF and PVR values measured during apneic periods throughout the 6-h recording period were averaged. All values from each fetus were then grouped, and a mean ± SE was calculated to provide an estimate of the normal variability of PBF and PVR that can occur daily during apneic periods.

The changes in PBF, PVR, systemic arterial pressure (SAP), and Ppa between control and TO fetuses during periods of apnea were compared over the 8 experimental days using a two-way ANOVA with repeated measures. Changes in the waveform during intraluminal pressure equalization were compared using a two-way repeated-measures ANOVA. Data in the text are expressed as means ± SE. The level of significance was P < 0.05 for all statistical analyses.
RESULTS

Physiological Status of Fetuses

All fetuses were considered healthy according to their blood-gas and acid/base status, both before and during the experiment. There were no significant differences in any of the blood-gas parameters between control fetuses and fetuses exposed to 7 days of TO at any time point over the 8-day experimental period. Measured at 127 ± 2 days, the mean blood-gas tensions and pH values in fetal carotid arterial blood were as follows: pH 7.35 ± 0.01, arterial PCO2 52.1 ± 0.6 Torr, Pao2 20.6 ± 1.0 Torr, and arterial O2 saturation 58.9 ± 2.9%.

Similarly, fetal body weights were not different between control fetuses (2.70 ± 0.31 kg) and fetuses exposed to 7 days of TO (3.05 ± 0.25 kg). However, fetal lung wet weights were significantly greater in TO fetuses compared with controls (55.1 ± 4.8 vs. 35.1 ± 4.6 g/kg; P < 0.05), as was total lung DNA content (237.9 ± 11.1 vs. 170.7 ± 18.9 mg/kg; P < 0.05). Basal intraluminal pressures, measured during apneic periods, were significantly increased from 1.9 ± 0.8 mmHg before the onset of TO to 5.0 ± 0.9 mmHg after 7 days of TO; basal intraluminal pressure was not significantly altered in control fetuses throughout the experimental period.

Pulmonary Hemodynamics

Pulmonary hemodynamics during apnea. Before the onset of experiments, basal hemodynamic parameters were not different between control fetuses and fetuses destined to be exposed to TO. These include intraluminal pressure (1.3 ± 0.5 vs. 1.5 ± 0.3 mmHg), PBF (20.8 ± 4.2 vs. 20.9 ± 6.3 ml/min), PVR (2.3 ± 0.1 vs. 1.8 ± 0.5 mmHg·ml⁻¹·min⁻¹), PAP (32.6 ± 0.7 vs. 33.6 ± 1.0 mmHg), SAP (31.8 ± 0.6 vs. 32.3 ± 0.8 mmHg), and HR (169.0 ± 15.5 vs. 169.3 ± 4.6 beats/min). All measurements were made during apnea.

In control fetuses, mean PBF through the left pulmonary artery during periods of apnea increased linearly with age from 19.4 ± 4.5 ml/min (9.4 ± 1.3 ml·min⁻¹·kg body wt⁻¹) on the first day of the experimental period to 34.01 ± 1.9 ml/min (14.7 ± 3.3 ml·min⁻¹·kg⁻¹) by day 7 (r² = 0.83; P < 0.025). Similarly, in fetuses exposed to 7 days of TO, mean PBF measured during apnea increased linearly from 24.9 ± 6.0 ml/min (11.3 ± 3.6 ml·min⁻¹·kg⁻¹) to 41.8 ± 4.7 ml/min (15.0 ± 1.6 ml·min⁻¹·kg⁻¹) at 7 days of TO (r² = 0.914; P < 0.001) (Fig. 1A). The gestational age-related increase in PBF was similar in control fetuses and fetuses exposed to 7 days of TO. When PBF was adjusted for differences in lung weight between control and TO fetuses (measured at autopsy on day 7), PBF was not different in control fetuses (34 ± 10 ml·min⁻¹·100 g⁻¹) and fetuses exposed to 7 days of TO (28 ± 3.2 ml·min⁻¹·100 g⁻¹; P = 0.33), despite the higher intraluminal pressures (5.0 ± 0.9 vs. 1.9 ± 0.8 mmHg) in TO fetuses.

Over the 7 experimental days, PVR was significantly reduced in both control (by 47.7 ± 5.8%) and TO (by 29.5 ± 15.0%) fetuses, and the rate of reduction was similar in both groups (Fig. 1B). On the other hand, Ppa and SAP (carotid arterial pressure) during apnea did not significantly change over the 8 experimental days in either control fetuses or fetuses exposed to TO. No significant difference in these parameters was detected between control and TO fetuses at any stage.
Fetal lung expansion alters pulmonary hemodynamics

In all six fetuses exposed to 7 days of TO, recordings were made of fetal “Valsalva-like” maneuvers, which are believed to be forced inspiratory attempts against a closed airway. These maneuvers were only present in TO fetuses and occurred at an average frequency of 46 per hour in the four fetuses examined; one fetus had as many as 73 maneuvers per hour. They resulted in an increase in intraluminal pressure of up to ∼15 mmHg, with a mean increase in intraluminal pressure of 6.7 ± 0.3 mmHg; the minimum increase that was considered detectable was 2 mmHg. The average duration of a Valsalva-like maneuver was 7.4 s, with some maneuvers lasting >20 s. During these Valsalva-like maneuvers, mean PBF was reduced by 17.5 ± 4.0% (7.1 ± 2.3 ml/min; range 0–38 ml/min) compared with the mean PBF measured before the maneuver. Similarly, the amplitude of the PBF waveform was reduced (by 5–35 ml/min; Fig. 5) during these maneuvers, whereas PVR was increased by 33.9 ± 7% (P < 0.05). Furthermore, a strong correlation (r² = 0.53, P < 0.001) was found between the percent change in mean PBF and the mean increase in P, (i.e., mean pressure amplitude of maneuver) associated with each Valsalva-like maneuver (Fig. 6A).

A strong correlation was found between the change in PBF and the change in PVR during Valsalva-like maneuvers (Fig. 6B), suggesting that the changes in PBF occurred because of an increase in PVR (r² = 0.89, P < 0.001). Ppa as well as carotid arterial pressure were both significantly increased during Valsalva-like maneuvers (7.8 ± 1.2 and 7.3 ± 1.3%, respectively; P < 0.05).

Equalization of Intraluminal Pressure

Hemodynamics during pressure equalization. In fetuses exposed to TO, the intraluminal pressure was reduced to values similar to that measured in control fetuses (~1–2 mmHg) on day 7 of TO; the remaining volume is still likely to be higher (in ml/kg fetal body wt) than in controls because of the larger size of the lung following TO (28). Following intraluminal pressure equilibration, mean PBF increased from 36.8 ± 5.2 ml/min to a maximum of 112.4 ± 22.7 ml/min (P < 0.05) within 15 min (Table 2; Fig. 7). This increase in mean PBF coincided with a decrease in PVR from 0.91 ± 0.14 to 0.32 ± 0.06 mmHg·ml⁻¹·min⁻¹. Maximum PBF increased from 331.1 ± 28.7 to 417.8 ± 8.3 ml/min, whereas minimum PBF

### Table 1. Mean PBF, PVR, Ppa, SAP, PBFmax, PBFmin, and heart rate during accentuated fetal breathing movements expressed as the percentage of the preceding apneic period (100.0%)

<table>
<thead>
<tr>
<th></th>
<th>0dTO</th>
<th>1dTO</th>
<th>2dTO</th>
<th>3dTO</th>
<th>4dTO</th>
<th>5dTO</th>
<th>6dTO</th>
<th>7dTO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean PBF</td>
<td>129.6±10.1*</td>
<td>116.5±3.9*</td>
<td>116.5±5.5</td>
<td>98.6±2.3</td>
<td>96.9±2.6</td>
<td>97.8±2.8</td>
<td>92.8±2.5*</td>
<td>93.9±2.1*</td>
</tr>
<tr>
<td>PVR</td>
<td>92.7±3.1*</td>
<td>94.3±2.1*</td>
<td>96.2±3.8</td>
<td>109.7±3.7</td>
<td>113.6±4.3</td>
<td>112.3±6.3</td>
<td>114.8±4.7*</td>
<td>114.0±6.3*</td>
</tr>
<tr>
<td>Ppa</td>
<td>105.6±1.3*</td>
<td>105.1±1.0*</td>
<td>102.8±1.3*</td>
<td>103.9±2.0*</td>
<td>103.9±1.5*</td>
<td>102.7±1.8*</td>
<td>102.2±2.4</td>
<td>100.3±2.0</td>
</tr>
<tr>
<td>SAP</td>
<td>105.1±1.0*</td>
<td>104.6±1.2*</td>
<td>102.9±1.3*</td>
<td>106.7±1.2*</td>
<td>105.3±1.1*</td>
<td>106.2±0.9*</td>
<td>105.7±1.4*</td>
<td>103.2±0.7*</td>
</tr>
<tr>
<td>Heart rate</td>
<td>103.6±0.8*</td>
<td>102.8±0.8*</td>
<td>103.9±0.9*</td>
<td>103.9±0.9*</td>
<td>103.5±1.1*</td>
<td>102.0±0.6*</td>
<td>105.1±1.6*</td>
<td>103.5±0.9*</td>
</tr>
<tr>
<td>PBFmax</td>
<td>99.05±0.4</td>
<td>100.4±0.4</td>
<td>99.3±0.6</td>
<td>97.2±0.7*</td>
<td>99.0±0.5</td>
<td>98.0±0.7*</td>
<td>98.2±0.6*</td>
<td></td>
</tr>
<tr>
<td>PBFmin</td>
<td>95.2±1.6*</td>
<td>96.3±1.8*</td>
<td>97.6±1.4</td>
<td>98.3±1.2*</td>
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<td>98.6±1.6*</td>
<td>101.1±2.7</td>
<td>98.2±1.3*</td>
</tr>
</tbody>
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Values are means ± SE in % of apnea. 0dTO–7dTO, day 0 tracheal obstruction (TO) to day 7 TO; PBF, pulmonary blood flow; PVR, pulmonary vascular resistance; Ppa, pulmonary arterial pressure; SAP, systemic arterial pressure; PBFmax, peak PBF; PBFmin, minimum PBF. *Significantly different (P < 0.05) from the preceding apneic period.
increased from \(-122.8 \pm 14.9\) to \(-61.4 \pm 20.1\) ml/min (Fig. 8). In one animal, negative or retrograde PBF was abolished completely by the pressure equalization procedure. Interestingly, there was no change in the amplitude of the PBF waveform, which suggests that the entire PBF waveform shifted in a positive direction because of parallel changes in peak and minimum PBF. Ppa and SAP did not significantly change during the pressure equalization procedure, whereas the fetal HR tended to increase from \(154.7 \pm 7.3\) to \(168.4 \pm 6.6\) beats/min at 15 min, although this increase was not statistically significant. Two hours after commencing the pressure equalization procedure, most variables had returned to values observed before the procedure.

Waveform analysis during pressure equalization. The characteristics of the PBF waveform were broken down into a number of parameters, as described previously (41). All waveform variables during systole, except peak systolic flow, were unchanged by intraluminal pressure equalization; these included pulse amplitude, rate of increase, and rate of decrease in PBF. However, the variables associated with diastole, or the relaxation phase of the cardiac cycle, were significantly altered following equalization of intraluminal pressure. In fetuses exposed to 7 days of TO, mean diastolic, end-diastolic, and maximum flow during diastole increased significantly within 15 min of pressure equalization (Fig. 8) as did end-systolic flow (Fig. 8; \(P < 0.05\)). The PI, a measure of small-vessel resistance to pulsatile flow (19), was significantly reduced within 15 min of intraluminal pressure equalization (Fig. 8, \(P < 0.05\)). Similarly, the net volume of blood passing through the left pulmonary artery per beat was significantly increased from \(0.26 \pm 0.04\) ml/beat before pressure equalization to \(0.71 \pm 0.11\) ml/beat (\(-278\%; P < 0.05\)) at 15 min after intraluminal pressure equalization. All PBF waveform parameters, except for mean diastolic flow, had returned to control values by 60 min after commencing the pressure equalization procedure.

DISCUSSION

We have examined the effects of prolonged increases in lung expansion on pulmonary hemodynamics in fetal sheep. In particular, we have investigated the effects of increased lung expansion on 1) the gestational age-related increase in PBF and PVR; 2) the relationship between FBMs and PBF and PVR; 3) the effect of Valsalva-like maneuvers on PBF; and 4) the effect of restoring the intraluminal pressure (by reducing the volume of lung liquid) upon pulmonary hemodynamics. Our findings provide further compelling evidence to indicate that intralumi-
nal pressure is a major determinant of PBF in the fetus by regulating PVR. Increases in pressure reduce PBF, whereas reductions in pressure increase PBF. This is supported by the finding that increases in $P_t$, associated with Valsalva-like maneuvers cause a large reduction in PBF. Furthermore, reductions in $P_t$ associated with accentuated FBMs increase PBF before TO, whereas, following TO, when $P_t$ changes associated with FBMs become positive (27), accentuated FBMs cause reductions in PBF. However, despite the prolonged rise in intraluminal pressure associated with TO, PBF gradually increased, and PVR gradually decreased, with increasing gestational age, in parallel with the increase observed in control fetuses. This result supports a recent study demonstrating that the flow/velocity profile for blood flow in the left pulmonary artery is not altered by prolonged TO, as measured by Doppler ultrasound (48). Moreover, at day 7 of TO, PBF adjusted for lung weight was not different from control values, despite a significantly higher intraluminal pressure. We suggest that this finding results from growth of the pulmonary vascular bed in response to TO, leading to an increase in the cross-sectional area and to a decrease in PVR.

It is well established that, in the mature lung, increases in alveolar pressure influence PBF during positive pressure ventilation. Increases in alveolar pressure reduce the capillary/alveolar transmural pressure, causing compression of perialveolar capillaries (16–18, 32, 45, 49, 52). On the other hand, reductions in alveolar pressure increase the transmural pressure, causing expansion of perialveolar capillaries and a decrease in PVR (18, 40). Similarly, a reduction in intraluminal pressure caused by lung deflation increases PBF and decreases PVR in late-gestation fetal sheep (25, 53). On the other hand, increases in lung expansion reduce PBF, increase PVR, and cause closure of blood vessels <300 μm in diameter at total lung capacity (53). It is of interest that, in the latter study, the $P_t$ associated with closure of small blood vessels and the cessation of PBF (i.e., at total lung capacity) was $-5 \text{ cmH}_2\text{O} \approx -3.7 \text{ mmHg}$). In the present study, mean $P_t$ was $5.0 \pm 0.9 \text{ mmHg} \approx 6.5 \text{ cmH}_2\text{O}$ on day 7 of TO, which would be expected to cause PBF to cease in control fetuses (53). How-

Fig. 5. Representative example of the change in the PBF waveform (A), tracheal pressure (B), and mean PBF (C) during a Valsalva-like maneuver in a fetus exposed to 7 days of TO. Note the change in amplitude of the PBF waveform and the associated decrease in mean PBF that occurs during the large increase in tracheal pressure (intraluminal pressure).

Fig. 6. A: significant correlation ($r^2 = 0.53, P < 0.001$) occurred between the amplitude in tracheal pressure change and mean percent change in PBF during Valsalva-like maneuvers ($>2 \text{ mmHg}$) in fetuses exposed to 7 days of TO. All measurements were made during apnea. B: the relationship between mean PBF and mean PVR during Valsalva-like maneuvers, expressed as percentage of the period immediately preceding the maneuver. Both relationships were determined from data obtained from 4 fetuses ($r^2 = 0.89, P < 0.001$).
ever, we found normal PBF, despite the high intraluminal pressure, indicating that the pulmonary vascular beds are different between the two groups. We believe that this difference is due to growth of the pulmonary vascular bed, as the increase in PBF and reduction in PVR were not associated with alterations in Ppa, SAP, or HR and are, therefore, unlikely to result from alterations in cardiac output.

Previous studies have shown that TO can reverse the high PI associated with pulmonary hypoplasia caused by a diaphragmatic hernia (47) and corrects the abnormal muscularization of pulmonary arterioles seen in congenital diaphragmatic hernia (31, 33). As the PI reflects pulmonary vascular impedance and is a measure of small-vessel resistance to pulsatile flow (8, 43), it was suggested that TO can reverse the changes in the pulmonary vasculature associated with pulmonary hypoplasia (31, 33, 47). These pathophysiological changes caused by congenital diaphragmatic hernia include a decrease in the size of the vascular bed, a decrease in the number of vessels per unit area, and increased muscularization of intra-acinar vessels (10, 11, 38). As a result, infants born with severe pulmonary hypoplasia usually have an elevated PVR and a persistent right-to-left shunt (38). Thus the findings of our study provide further evidence to indicate that prolonged alterations in fetal lung expansion have a significant impact on the growth and development of the pulmonary vascular bed. However, as the relationship between intraluminal pressure and PBF was found to markedly differ between control and TO-exposed fetuses, it is possible that the vascular bed is fundamentally different between the groups. This could arise due to vascular growth predominantly occurring in extra-alveolar vessels, allowing flow to bypass the alveolar capillaries. However, our finding that intraluminal pressure equalization markedly increases PBF in TO fetuses indicates that the pulmonary vascular bed remains sensitive to acute changes in intraluminal pressure in these fetuses.

It is also possible that the altered relationship between intraluminal pressure and PBF in TO fetuses is influenced by vasoactive mediators. However, studies examining the myogenic nature of the fetal pulmonary vasculature in response to vasodilation indicate that this mechanism is unlikely.

Table 2. Peak systolic PBF, PBF<sub>min</sub>, Ppa, SAP, and heart rate measured in fetuses exposed to tracheal obstruction before and at selected intervals after equalizing intraluminal pressure of the fetal lungs

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Peak systolic PBF, ml/min</th>
<th>PBF&lt;sub&gt;min&lt;/sub&gt;, ml/min</th>
<th>Ppa, mmHg</th>
<th>SAP, mmHg</th>
<th>Heart rate, beats/min</th>
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<td>5</td>
<td>331.1±28.7</td>
<td>-122.8±14.9</td>
<td>34.3±1.4</td>
<td>33.2±1.3</td>
<td>154.6±7.3</td>
</tr>
<tr>
<td>15</td>
<td>417.8±8.3*</td>
<td>-61.4±20.1*</td>
<td>33.9±1.2</td>
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<td>168.4±6.6</td>
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<tr>
<td>30</td>
<td>412.5±32.5*</td>
<td>-61.1±15.5*</td>
<td>33.3±1.3</td>
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<td>169.4±6.0</td>
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<tr>
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<td>-83.6±12.2*</td>
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<tr>
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<td>33.3±1.8</td>
<td>32.2±1.5</td>
<td>152.8±4.1</td>
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Values are means ± SE. *Significantly different (P < 0.05) from the value before equalization of intraluminal pressure (5 min).

Fig. 7. Mean PBF (○) and PVR (●) before (−5 min) and during a 2-h period of intraluminal pressure equalization in fetuses exposed to 7 days of TO.

Fig. 8. Effect of equalizing the intraluminal pressure of fetal lung liquid in fetuses exposed to 7 days of TO on end-systolic (A), end-diastolic (B), and mean diastolic (C) PBF and pulsatility index (PI; D). The later is derived by the formula $PI = \frac{\text{peak systolic flow} - \text{minimum diastolic flow}}{\text{mean maximum flow averaged over several cycles}}$. Values that do not share a common letter are significantly different from each another, P < 0.05.
stimuli that increase fetal PBF, including increased PaO₂, shear stress, and several pharmacological agents, only have transient effects (1–4). Thus, if vasodilation was responsible for the maintenance of PBF during TO, when intraluminal pressures are elevated, we would expect the response to be transient and would not be maintained for the full 7 days of TO.

Our laboratory has previously demonstrated that PBF significantly increases during accentuated FBMs, which was attributed to phasic reductions in intraluminal pressure, leading to phasic increases in the capillary/alveolar transmural pressure (41). Our present findings confirm and extend these observations. We have shown that, over the first day of TO (day 0–1), PBF was significantly increased (by ~30%) during accentuated FBMs, compared with the preceding period of apnea. This is reduced compared with that observed in control fetuses (41). However, by day 7 of TO, the effects of accentuated FBMs on PBF had reversed, causing a significant decrease in PBF and increase in PVR. This “reversal” of the effect of accentuated FBMs on PBF occurred between days 2 and 3, was significant on day 5 of TO (see Fig. 2), and is thought to result from eversion of the fetal diaphragm (27, 35). Our laboratory has previously demonstrated that, following prolonged TO, the expanding lung causes caudal displacement of the diaphragm, which eventually everts after ~4–5 days of TO (27, 35). As a result, diaphragmatic contractions associated with FBM cause compression of the lungs, which is illustrated by the reversal of the Pt fluctuations, which change from negative to positive (Ref. 27; see Fig. 1). Thus we consider it likely that, during accentuated FBMs, contraction of the everted diaphragm causes phasic compression of the lung, which increases intraluminal pressure and compresses the perialveolar capillaries, leading to an increase in PVR. Indeed, the time course of the change in the relationship between accentuated FBM and PBF during TO was very similar to the time course for the reversal in pressure fluctuations associated with FBM.

It is possible that a failure to increase right ventricular output during accentuated FBMs on days 6 and 7 of TO may have contributed to the decrease in PBF during accentuated FBMs, as no significant increase in Ppa was detected during accentuated FBMs at this time. However, we consider this unlikely, as the highly significant relationship between PBF and PVR during accentuated FBMs was identical on day 0 of TO to that observed on day 7 of TO (see Fig. 4), indicating that changes in PVR are likely to be the predominant contributing factor at both time points. However, as we did not measure Pla (assumed to be 3 mmHg), our calculated values of PVR may slightly overestimate the true changes in PVR, if Pla increased during accentuated FBMs.

Following TO, all fetuses examined made regular Valsalva-like maneuvers, but it is possible that lung volume receptors (pulmonary stretch receptors) detected high lung volumes and initiate this maneuver via vagal afferent feedback in an attempt to reduce them (9). The close correlation between the increase in intraluminal pressure and the decrease in PBF provides further compelling evidence to support the concept that intraluminal pressure is a major regulatory factor of PBF in the fetus. Indeed, the reductions in PBF were tightly constrained to the periods of increased pressure, suggesting that the effect on the pulmonary vascular bed is mediated by direct compression rather than the release of a vasoconstrictor substance. Despite the increase in PAP and SAP, it is likely that cardiac output (not measured) was reduced due to a decrease in venous return, as has been classically described in the adult (21, 22), or to a reduction in Pla due to the high intraluminal pressure generated.

To account for the differences in intraluminal pressure between control fetuses and fetuses exposed to TO, we reduced the intraluminal pressure in TO animals by draining the lungs of liquid until the intraluminal pressure was equivalent to that observed in control fetuses (1–2 mmHg). Within 15 min of commencing this procedure, PBF had increased by ~300%, and PVR had decreased by ~60%, presumably in response to the associated reduction in intraluminal pressure. This was associated with a vertical shift in the PBF waveform, resulting in a reduction in retrograde flow during diastole of ~50%. In one fetus, retrograde flow during diastole was abolished (mean minimum PBF = 7 ml/min), indicating that forward flow occurred throughout the cardiac cycle. The very large increase in PBF associated with pressure equalization in TO fetuses is consistent with the suggestion that the growth response of the lung to TO includes marked growth and development of the pulmonary vascular bed, leading to a marked decrease in PVR for any given intraluminal pressure. However, within 2 h of pressure equalization, most parameters (other than mean diastolic flow) had returned to values observed before the drainage, despite intraluminal pressures remaining low. The reasons for this return to the predrained values are unknown, although it is possible that the large initial increase in PBF elicited a shear stress-mediated vasoconstriction (29). It would be interesting, therefore, to determine whether this effect is of an acute nature or is sustained.

Following intraluminal pressure equalization, the major changes in the PBF waveform occurred during diastole, with little change in the characteristics associated with systole; these changes are similar to those that occur at birth (40). During diastole, PBF is largely determined by PVR, which, in the fetus, is responsible for generating the backward traveling compression and expansion waves, which reflect off the pulmonary vascular bed, causing retrograde flow (20); other factors include the elastic recoil of the main pulmonary arteries as well as flow through the DA. As intraluminal pressure equalization markedly reduced PVR, it is not surprising that the PBF waveform characteristics most sensitive to this procedure occur during diastole when PVR is the dominant factor. This finding provides further evidence to indicate that certain components of the PBF waveform, particularly during diastole, can act as sensitive indicators of PVR in the presence of a patent DA.

In summary, our study demonstrates that PBF is maintained during TO in the fetus, despite the sustained increase in

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intrapulmonary pressure, and provides further evidence to indicate that intrapulmonary pressure is an important determinant of PBF in the fetus. Our findings suggest that the fetal lung growth response to TO includes growth of the pulmonary vascular bed, which likely explains the normal gestational age-related increase in PBF in the presence of elevated intrapulmonary pressures. Indeed, reducing the intrapulmonary pressure to control values after 7 days of TO caused a marked increase (~300%) in PBF, suggesting that the cross-sectional surface area of the pulmonary vascular bed had increased. When combined with our laboratory’s previous findings (41), our findings on the pulmonary vascular bed had increased. When combined with our laboratory’s previous findings (41), our findings on the effect of intraluminal pressure equalization on PBF following 7 days of TO, provides compelling evidence to indicate that intrapulmonary pressure is a major regulatory factor determining fetal PBF.

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GRANTS

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